RESEARCH NOTE

Is Vigor of Regeneration a Key Factor in Recovery from Peripheral Nerve Injuries?

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The nerve-growth-promoting effects of the tricyclic antidepressant, imipramine, were tested on the sympathetic ganglion of chickens and on the sciatic nerve of rats. A powerful neuronotrophic action was observed *in vitro*, but the utilization of the drug *in vivo* did not modify the functional recovery from a crush lesion. © 1986 Academic Press, Inc.

Le Gros Clark's observation that nerve fiber regrowth is enhanced by salivary gland tissue (7) was followed by Bueker's work with mouse sarcoma tissue (1). Since then, nerve growth factor (NGF) has been used experimentally in both central and peripheral nervous systems. The *in vivo* effects of NGF, however, are difficult to analyze because it is quickly metabolized. Therefore, there is no answer to the following questions: (a) In order to obtain optimal enhancement of regeneration, should NGF be applied: to the cell body? to the neurite at the site of damage? or distally, with the idea of attracting and guiding the sprouts? (b) Would the increased vigor of regeneration thus produced by useful in clinical practice?

Recently, the tricyclic antidepressant, imipramine, was found by one of us (T.Q.) to have a strong NGF-like effect *in vitro*. Briefly, dissociated neurons from sympathetic ganglia of 12-day-old chick embryos were incubated 24 h in the absence (control), or in the presence of 1 μm imipramine. The number of surviving neurons was determined by direct double-blind count. The

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number of surviving neurons per milliliter was almost doubled in the presence of imipramine (Tam Quach, in preparation).

In the present study, we tested the behavioral effects of imipramine on the peripheral nervous system. Imipramine is relatively slowly catabolized and, when injected intraperitoneally, diffuses until even distribution is attained throughout the body. By insuring a long-term action of the drug all along the nervous tracts, this circumvented the first of the above questions.

Sprague–Dawley male rats weighing 200 to 250 g at the beginning of the experiment were anesthetized with pentobarbital and chloral hydrate, i.p. In all animals, the left sciatic nerve was aseptically exposed and crushed at midthigh with maximum force for 1 min in the bare jaws of a serrated hemostat. The resulting lesion was 2 mm wide. In 19 animals, 2 mg/kg of imipramine were injected daily intraperitoneally; the treatment started 3 days before the procedure, and was continued until the 25th postoperative day. In another group of 18 rats, only solvent was injected.

The functional condition of the rats was assessed through the use of the sciatic functional index (SFI), based on measurements made from rats' walking tracks and expressed in units of functional deficit (3). Collection and analysis of data were made by a "blind" observer with a data management system (4). Animals were tested preoperatively and at regular postoperative intervals.

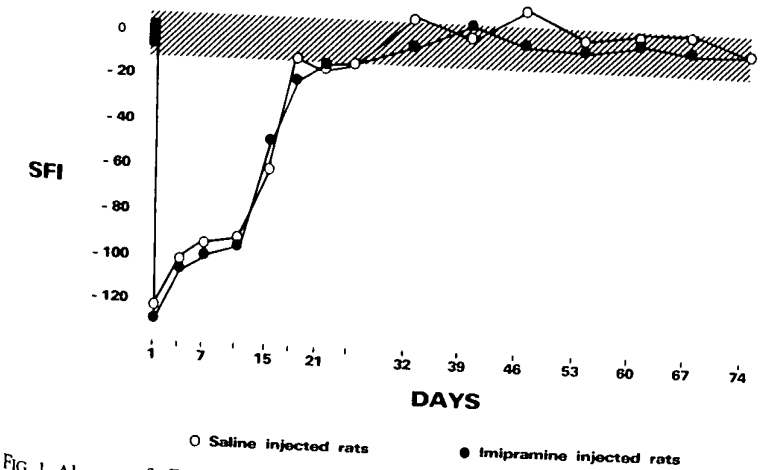

FIG. 1. Absence of effect of imipramine on the functional recovery from a crush lesion to the sciatic nerve of the rat. The mean (±SE) sciatic functional index expressed in units of deficit is shown as a function of time. The shaded area between +10 and −10 defines normality. Functional recovery was identical in both groups.

No difference was found in functional recovery of the two groups (Fig. 1); in all animals, recovery commenced at about day 15 and was satisfactory by day 21.

The results obtained for the period of functional recovery (days 11 through 25) were submitted to a one-way multiple analysis of variance (MANOVA). The test was not significant from day 11 [$F(1,36) = 0.47$, $P = 0.49$] through day 25 ($F = 0.00$, $P = 0.96$), confirming that imipramine had no clinically detectable effects on sciatic nerve regrowth.

Three interpretations could be offered for the lack of efficacy of imipramine *in vivo:* (a) Imipramine may act exclusively on sensory neurons. Evaluation of sensory levels in animals is notoriously imprecise (2), and our method of functional assessment could be insufficient to demonstrate the beneficial effects of the drug. The general reliability of this method, however, has been independently confirmed (5, 6, 8). (b) The effects of increased vigor of regeneration might be difficult to measure in the rat because of the short distance the sprouts have to grow before reaching their target. Moderate and short-lived differences could thus be overlooked. Over greater distance, i.e., in longer nerves, differences would be magnified, last longer and become measurable. (c) Vigor of regeneration might not be a key factor in the recovery from peripheral nerve injuries. We believe that this last interpretation is correct; the unpredictable functional results of peripheral nerve surgery can be explained without reference to growth promoting substances (L. de Medinaceli, in preparation).

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RESEARCH NOTE

Angiotensin II-Sensitive Neurons in the Rat Lateral Hypothalamic Area with Efferent Projections to the Subfornical Organ

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Thirteen neurons in the lateral hypothalamic area were antidromically activated by electrical stimulation of the subfornical organ in the rat. The activity of almost all identified neurons ($N = 11$) was excited by microiontophoretically applied angiotensin II. On the other hand, of the antidromically unidentified lateral hypothalamic area neurons ($N = 10$) tested, 4 were excited by microiontophoretically applied angiotension II and 6 were not affected. The excitatory responses of identified and unidentified lateral hypothalamic area neurons to angiotensin II were blocked by microiontophoretically applied saralasin, an angiotensin II antagonist.

The subfornical organ (SFO) is implicated in angiotensin II (AII)-induced drinking (1, 7, 10, 14), a centrally mediated pressor response (4, 7, 10), and the control of vasopressin release from the neurohypophysis (3, 6). Recent immunohistochemical studies have identified AII-immunoreactive pathways from the lateral hypothalamic area (LHA) to the SFO (8, 9), suggesting the importance of these pathways for the control of body fluid balance and central cardiovascular regulation.

In a previous study (5), we described that LHA efferent fibers have an excitatory influence on the activity of SFO neurons projecting to the supraoptic nucleus. Because the LHA receives the AII-immunoreactive effer-

Abbreviations: LHA—lateral hypothalamic area, SFO—subfornical organ, MIPh—microiontophoretically, AII—angiotensin II, Sar—saralasin.

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